EXPERIMENT K-6-19

PINEAL PHYSIOLOGY IN MICROGRAVITY: RELATION TO RAT GONADAL FUNCTION

Principal Investigator:

D. Holley
Department of Biological Sciences
San Jose State University
San Jose, California 95192

Co-Investigators:

M.R.I. Soliman
College of Pharmacy and Pharmaceutical Sciences
Florida A & M University
Tallahassee, Florida 32307

F. Kaddis
College of Pharmacy and Pharmaceutical Sciences
Florida A & M University
Tallahassee, Florida 32307

C. Markley
Department of Biological Sciences
San Jose State University
San Jose, California 95192

I. Krasnov
Institute of Biomedical Problems
Moscow, USSR

INTRODUCTION

Relative to other endocrine organs, research on the physiology of the pineal has been a rather recent endeavor, and discoveries relative to pineal physiology have proceeded rather slowly. Considerable advances in this area have occurred over the past two decades and many textbooks are now espousing it as a true endocrine gland (Hadley, 1988). It is now known that the pineal organ can interact with many endocrine and nonendocrine tissues in a regulatory fashion. It is well established that an antigonadal hormone of pineal origin (most likely the indoleamine melatonin) is involved in the photoperiodic regulation of reproduction in seasonally breeding mammalian species, and some nonseasonal breeding species (possibly also humans). Recent reviews implicate the pineal in the functioning of other organs and systems besides those involved in reproduction including: temperature regulation, thyroid, growth hormone, adrenal glucocorticoid synthesis, behavior (arousal/depression), circadian system (activity/rest), and skin pigmentation (in lower animals). Several excellent journal reviews and books on pineal physiology have appeared over the past ten years (see Reiter, 1981a; Reiter, 1981b; Vollrath, 1981; Reiter, 1982; Binkley, 1983; Axelrod, et al. 1983; Preslock, 1984; Reiter, 1984; Brown and Wainwright, 1985; O'Brien and Klein, 1986). In view of the fact that the pineal is an important link to the environment (Reiter, 1986), it is conceivable that exposure to microgravity and spaceflight might alter the function of this gland and, in turn, affect various physiological functions including the circadian timing system and reproduction.

Primary control of pineal function is mediated by the photoenvironment. Light impinging upon the retina influences the pineal via the following pathway: retino-hypothalamic tract, suprachiasmatic nuclei, median forebrain bundle, superior cervical ganglia, sympathetic efferents. Adrenergic receptor activation results in stimulation of N-Acetyltransferase (NAT) activity with resulting production of melatonin from the precursor serotonin (5- hydroxytryptamine, 5-HT). Indeed, the serotonin concentration of the pineal gland exceeds that of any other organ and is fifty times that of any other brain area (Quay, 1963). Melatonin is probably the most important pineal secretory product in terms of distant regulatory responses (e.g. antigonadal effects). However, several other non-indole hormones are reputed to be synthesized there, (e.g., arginine vasotocin, oxytocin, arginine vasopressin, an alpha-MSH like peptide, GnRh, TRH, renin, and angiotensin I). The pineal melatonin content fluctuates with a pronounced circadian rhythm (amplitude of about 20-plus orders of magnitude) and it is rapidly inhibited when the animal is exposed to light. Wurtman and Ozaki (1978) suggested that the availability of serotonin may be involved in regulating the synthesis of melatonin in the pineal. Chan and Ebadi (1980) provided evidence that under certain experimental conditions serotonin may inhibit the activity of NAT, a key enzyme in the synthesis of melatonin. Serotonin also exhibits circadian rhythmicity (amplitude of approximately 2-3 orders of magnitude) and this rhythm persists even in blinded weanling rats. Given its key role in the regulation of melatonin synthesis, its high concentration, and that its levels may persist longer than the more rapidly changing melatonin, we felt that serotonin might give a more accurate assessment of the effects of microgravity on pineal function following recovery of the animals from the flight. We also measured 5- hydroxyindole acetic acid (5-HIAA), a major metabolite of serotonin, hoping that we might be able to assess an effect on serotonin metabolism (turnover).

One of the most interesting concomitants to spaceflight and exposure to microgravity has been the disturbing alteration in calcium metabolism and resulting skeletal effects. It was recognized as early as 1685 (cited in Kitay and Altschule, 1954) that the pineal of humans calcified with age. However, little can be found in the literature relating calcification and pineal function. Given the link between exposure to microgravity and perturbation of calcium metabolism and the fact that the pineal is apparently one of the only "soft tissues" to calcify, we examined pineal calcium content following the spaceflight.

MATERIALS AND METHODS

Cosmos 1887 animal groups

a) Flight animals.

Pineals were obtained from 5 (#6-#10) of the 10 male rats (Czechoslovakian- Wistar, origin Institute of Endocrinology, Brattislava, Czechoslovakia) that were flown aboard the Soviet Biosatellite Cosmos 1887. The flight launched 9/29/87 at 15:50 and landed 10/12/87 at 07:03 (Moscow time). The orbital inclination was 62.8 degrees and the apogee and perigee were 406 and 224 kilometers, respectively. Fourteen gram boluses of food (total 55 g/ rat/ day) were provided at 02:00, 08:00, 14:00, and 20:00 hrs. each day. Water was provided ad libitum. The air pressure in the cage was 760 mmHg, the humidity averaged 58%, and the ambient temperature was 22-23 degrees C. Lights were on from 08:00-24:00, and off from 24:00-08:00. Light intensity was 4-8 lux at the cage floor and was provided by an incandescent lamp placed over each feeder. Due to problems related to the recovery of the biosatellite once it had landed, the final sacrifice and dissection occurred on the morning of 10/14/87, approximately 49 hours after the re-entry landing. The animals were last fed in flight at 02:00 hrs. on 10/12/87, and were not fed again until in the animal quarters at the recovery/dissection site at 20:00 hrs on 10/13/87 when they received a half day portion of food (28 g/ each).They were, therefore, without food for approximately 42 hours. Given the logistics of the recovery and the fact that the lights were turned on in the satellite at 05:00 hrs. on 10/12/87 in preparation for the landing, the light cycle was not constant. From data provided regarding the post flight recovery it is apparent that the animals experienced about a 3-5 hour phase advance and were subjected to a 36 hour day (20 hours darkness : 16 hours light) upon landing.

b) Basal control animals.

They were put into flight-type cages and had a flight (paste) diet for 14 days before sacrifice on 09/24/87. Temperature, humidity, and lighting were similar to in-flight conditions.

c) Synchronous control animals.

These rats were maintained in flight-type cages on a flight (paste) diet. They were exposed to the launch G forces and vibration, deprived of food for 42 hrs. and exposed to the same lighting regimen and temperature as flight rats after landing. After their "simulated flight" sacrifice was delayed the same period as for flight rats. The re-entry G force and post flight transportation conditions of the flight animals were not mimicked for the synchronous controls.

d) Vivarium control animals.

These animals were kept in cages of the same size as the flight cages with environmental conditions similar to those in flight. They were fed the same quantity of food per day (55 g) but in only one feeding. On the night prior to sacrifice, food was withdrawn from these animals at 20:00 hrs. Post flight conditions (e.g. temperature) were not mimicked for this group.

Sample collection and initial tissue extraction step.

Upon dissection the pineals were immediately placed in prechilled cryovials (10 x 55 mm), placed into liquid nitrogen for quick-freeze, and shipped to Moscow in a liquid nitrogen biotransporter. The samples were shipped to the U.S.A. and stored until analysis at -70 degrees C. Trunk blood was collected immediately following decapitation into heparinized tubes and centrifuged at 4 degrees C. Plasma (1.5 ml) was rapidly frozen in liquid nitrogen and shipped to the U.S.A. in a

biotransporter. In the U.S.A. this volume was thawed and aliquoted (100 µl) for distribution to various investigators.

The pineal glands were thawed at room temperature, weighed, and homogenized in 200 µl of perchloric acid. 100 µl of the crude homogenate was frozen and saved for the calcium analysis. The remaining aliquots (100 µl) were immediately used for the HPLC analysis of 5-HT and 5-HIAA.

HPLC analysis of pineal serotonin, 5-HIAA, and plasma.

Aliquots (100 µl) of the crude pineal homogenate were centrifuged using Centrex brand microfilters (0.45 um pore size, Schleicher and Schuell, Inc., Keene, New Hampshire, U.S.A.). Homovanillyl alcohol was added to the homogenates to act as an internal standard at a final concentration of 10^{-6}M. Serotonin and 5-HIAA were analyzed in the filtered homogenates by HPLC using a modification of the method of Medfford and Barchas (1980). The filtered homogenates were injected into a u-Bondapack brand C-18 reverse phase column of a high pressure liquid chromatograph (Bioanalytical Systems, Inc.). The mobile phase consisting of 0.1 M sodium acetate, 0.1 M citric acid and 25% v/v methanol (pH 4.1) was run through the column at a flow rate of 1.0 ml/min. The various peaks were detected using an electrochemical detector (Bioanalytical Systems, Inc.) mounted with a glassy carbon working electrode and Ag/AgCl reference electrode set at an oxidation potential of 0.85 V and sensitivity of 10 nAmps/V. Peaks were integrated and analyzed using a Bioanalytical Systems, Inc. workstation mini-computer.

For analysis of plasma 20 µl of 1.0 M perchloric acid was added to 100 µl of plasma. The mixture was filtered using micro-filter centrifuge tubes (Centrex brand, see above). The filtrate (20 µl) was injected onto the HPLC column as above.

Radioimmunoassay of pineal melatonin content.

The melatonin content of the pineal homogenates (75 µl aliquots) were determined by radioimmunoassay using "ultraspecific" melatonin antiserum and a procedure provided by Dr. G. Brown (CIDtech Research, Inc., Ontario, Canada) using ^{3}H-melatonin (Amersham Corp., Arlington Heights, IL, U.S.A.). The melatonin for standard was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. (cat, #M5250). The assay performance characteristics in our laboratory were: sensitivity (defined as three standard deviations from the counts for the zero reference standard tube), 5 pg/ml; interassay coefficient of variation, 11.5%; and intraassay coefficient of variation, 6.27%.

Atomic absorption analysis of pineal calcium content.

Total calcium content of the pineal homogenates was determined by atomic absorption spectrophotometry using an electrothermal atomizer equipped with a carbon rod. Aliquots (5 µl) of the homogenates were diluted with ultra-pure water (7 mohm resistance, Multi-Q Water System, Millipore, Corp., Bedford, MA, U.S.A.). Volume added was 200 or 250 µl to achieve absorbance values in the range 0.1-0.5 absorbance units. Calcium reference standard was obtained from VWR, Inc., San Francisco, CA, U.S.A. (cat. #EM-CX0082-1). Assay sensitivity was approximately 4 pg/µl.

Statistical analysis.

Given the experimental design, the groups were analyzed by one-way analysis of variance (ANOVA). If the ANOVA indicated a between group difference ($p < 0.05$), then the data were

further analyzed by one or more of the following statistical tests: Duncan's Multiple Range Test, Newman-Keul's Multiple Comparison Test, or Fisher's Least Significant Difference Test. In certain instances, Student's t-test for nonpaired samples was applied. The 95% confidence limit was considered significant in all tests.

RESULTS

The results of this study are summarized in Tables 1-3 and Figures 1-6.

A) Organ weights.

The data (Table 1) indicate that the flight rats were significantly smaller than the vivarium and synchronous control animals ($p < 0.01$). Due to this difference, testes and adrenal weights were normalized to percent of the body weight for group comparisons. Though the average testes weight of the flight group was less than the other two groups ($p < 0.01$ vs. vivarium), when normalized to percent body weight the differences were not significant. The pineal weights among the groups were also not significantly different. Adrenals of the flight group, however, were significantly enlarged when compared to the vivarium, synchronous and basal controls ($p < 0.001$), even for the normalized data. The adrenal enlargement is consistent with chronic exposure to one or more environmental stressors. It should be noted, however, that at time of sacrifice there was no statistical difference in the plasma corticosterone concentrations: Flight = 15.06±9.31 S.D.; Synchronous = 10.92±5.05; Vivarium = 22.10±13.67; Basal = 26.20±11.30 (data courtesy of Dr. R. Grindeland and Marilyn Vasques, NASA-Ames Research Center).

B) Pineal gland analysis.

Pineal melatonin content was determined for individual glands and the values normalized and reported as pg/milligram pineal tissue (see Table 2). There were no significant differences among the three test groups as determined by one-way analysis of variance. The results are summarized in Figure 1.

Serotonin (5-HT) and 5-HIAA content were also determined for individual glands and the values normalized and reported as ng/milligram pineal tissue (see Table 2). Results from two of the animals (F-9 and S-6), however, were considerably different from other values within their respective groups. Accordingly, if these values are included in a one-way analysis of variance, then there are no significant differences among the group means (see Figures 3 and 5). However, if the two values are removed from the analysis, then both flight group serotonin (5-HT) and 5-HIAA are significantly greater than controls (see Figures 4 and 6).

Table 2 summarizes the pineal calcium determinations. Two of the samples that had extremely high calcium concentrations were assumed to be contaminated, and were excluded from the analysis (V-6 and S-10). One-way analysis of variance indicated no statistical differences among the groups.

C) Plasma serotonin (5-HT), 5-HIAA and testosterone concentrations.

Table 3 indicates that the plasma testosterone concentration of the flight animals was lower than all the other groups. The difference, however, was significant only for the basal controls vs. the flight, vivarium and synchronous groups ($p < 0.01$). Note the difference between the flight versus basal group was highly significant ($p < 0.001$).

The plasma concentration of 5-HIAA in all groups was below the detectable sensitivity of the HPLC machine used in the analysis of the samples which indicates the plasma concentrations were less than 2.0 ng/ml.

Table 3 summarizes the plasma serotonin (5-HT) data. Three samples were lost in the extraction/ultrafiltration processing and were not included in the analysis (F-10, V-7, and S-7). Subsequent analysis revealed that the flight group had relatively lower plasma concentrations of 5-HT. The difference was not significant when subjected to one-way analysis of variance. However, the flight group was significantly different from the basal group when analyzed by Duncan's Range test, Fisher's LSD test and Student's t-test ($p < 0.05$). It should also be noted that the synchronous control group differed from the basal control group ($p < 0.05$, Fisher's LSD Test).

DISCUSSION

Considerable evidence supports the existence of a pineal humoral factor or factors (most likely melatonin) that can influence the hypothalamic-pituitary gonadal axis in many vertebrate species (Vollrath, 1981) including the rat (Binkley, 1983). If indeed the pineal is a major "link to the environment" (Reiter, 1986) it is, therefore, possible that gonadal function of rats flown in space might be altered via a mechanism that includes involvement of the pineal.

Several pieces of evidence indicate that gonadal function of the rats aboard the Cosmos 1887 spaceflight may have been compromised. Though the testes weights of the flight animals were lower than the synchronous and vivarium controls ($p < 0.01$), the body weights of the flight animals were also lower than the other groups (Table 1). When the testes weights were normalized as % body weight, the testes weight difference was not born-out. Plasma testosterone concentration, however, was lowest in the flight animals and was significantly lower than the basal control group ($p < 0.001$). Microscopic examination of the testes also revealed that there were 4% fewer spermatogonial cells in the flight group versus the vivarium control group ($p < 0.02$, data provided by Dr. D. Philpott).

It is known that light has a dramatic and rather immediate inhibitory effect upon rat pineal N-acetylserotonin and pineal melatonin levels (Binkley, 1983). As a result we felt that to assess pineal function we would have to measure parameters that were less labile. Green, et.al. (1977) indicated that potent stressors (e.g. electroconvulsive shocks) could alter serotonin turnover for up to 6 days post exposure. Since serotonin is an important precursor to melatonin we felt that its measurement and relative inertia with respect to relative changes in concentration might be used as an indirect indicator of melatonin synthesis. To do this, however, we felt measurement of 5-HIAA, a major serotonin metabolite, would be necessary as an indication of serotonin turnover.

As suspected, the pineal melatonin levels were very low at time of sacrifice (Table 2 and Figures 1 and 2). Given that the rats were sacrificed about 2 hours after lights on, and that the major circadian drop in circulating melatonin is locked to the time of lights on (Binkley, 1983), it is not surprising that the pineal melatonin content was low in all groups. Given the limited amount of plasma available to us for the analysis of 5-HT and 5-HIAA (100 µl), and given the low pineal melatonin levels, we did not measure the plasma melatonin concentration.

Figures 4 and 6 represent the 5-HT and 5-HIAA data after elimination of two statistical outliers. Note that exposure to the space environment resulted in a significant increase ($p < 0.05$) in the pineal levels of these substances. The pineal gland content profile was not reflected in the blood, however. Table 3 indicates that the flight and synchronous groups had low levels of 5-HT compared to the basal group ($p < 0.05$) and that all groups had undetectable levels of 5-HIAA. It is known that the plasma 5-HT levels may reflect peripheral secretion and may not be an accurate indicator of central serotonergic mechanisms. The results indicate that exposure to the space environment had an effect on the level of 5-HT and its turn-over as indicated by concomitant increase in 5-HIAA. This would be consistent with increased melatonin secretion during the spaceflight which may have been involved in the antigonadal activity noted.

It has been suggested that melatonin may be a hormone of "stress" given its increased secretion during conditions such as insulin-induced hypoglycemia (Wurtman and Moskowitz, 1977). Yet the melatonin circadian rhythm is 180 degrees out of phase with the corticosterone circadian rhythm and there is evidence for a direct inhibitory role of the pineal and melatonin on adrenal glucocorticoid synthesis (Ogle and Kitay, 1978). The adrenal hypertrophy in the flight animals would indicate a chronic stress response. However, at time of sacrifice the corticosterone levels were not statistically different among the groups. Either the conditions resulting in corticosterone secretion had dissipated by the time of sacrifice or the secretory response may have been inhibited (normal circadian nadir, or some other inhibitory factor or factors). The light cycle alterations (3-5 hour phase advance, and the 36 hour day (20 hours dark:16 hours light) imposed prior to sacrifice further complicate the interpretation of the data. Also, fasting is known to induce a hormonal secretory pattern similar to application of an environmental stressor (there was an approximately 4[illegible] hour fast during the recovery and sacrifice).

The pineal of humans and some other mammalian species contain multi-layered hydroxyapatite concentrations called corpora arenacea, or brain sand. Although the degree of radiologically detectable calcification of the human pineal gland appears to increase with age, there is no indication that increased calcification is related to loss of cellular activity. Histological and biochemical studies have shown that the appearance of the pinealocyte cell type, pineal serotonin content and HIOMT enzyme activity do not change with age (Giarman, 1960; Rodin and Overall, 1967; Smith, et al. 1977; Wurtman, 1964). Lukaszyk and Reiter (1975), suggest that the deposition of calcium may be related to polypeptide secretion by the pineal gland, and may serve as an index of previous glandular activity rather than degeneration. We found no statistical difference in the total pineal calcium content among our test groups. Though two values assayed extremely high, we felt that these were due to exogenous calcium contamination and were not included in the final analysis.

In summary, we conclude that the spaceflight resulted in a stress response as indicated by adrenal hypertrophy, that gonadal function was compromised, and that the pineal may be linked as part of the mechanism of the responses noted.

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TABLE 1. BODY WEIGHT, TESTES WEIGHT, AND PINEAL WEIGHT OF ANIMALS ON COSMOS 1887.

Group-Subj.#	Body Wt.(g)	Pineal Wt.(mg)	Testes wt.(g)	Testes Wt.(%B.W.)	Adrenal Wt.(g)	Adrenal Wt.(%B.W.)
F-6	304	1.51	1.24	0.41	48	15.8
F-7	306	1.90	1.25	0.41	54	17.7
F-8	296	1.38	0.85	0.29	54	18.2
F-9	300	2.47	1.30	0.43	44	14.7
F-10	310	1.45	1.32	0.43	54	17.5
X	303.2[a,b]	1.74	1.19[a]	0.39	50.8	16.78[d,e,f]
S.D.	5.40	0.45	0.19	0.06	4.60	1.47
S.E.M.	2.42	0.20	0.09	0.03	2.06	0.66
V-6	350	1.62	1.35	0.39	43	12.3
V-7	355	1.92	1.57	0.44	47	13.2
V-8	315	1.84	1.42	0.45	40	12.7
V-9	355	2.11	1.45	0.41	39	11.0
V-10	335	2.24	1.40	0.42	44	13.1
X	342.0	1.95	1.44	0.42	42.6	12.66
S.D.	17.13	0.24	0.08	0.02	3.21	0.89
S.E.M.	7.68	0.11	0.04	0.01	1.44	0.40
S-6	345	1.86	1.30	0.38	39	11.3
S-7	365	1.75	1.42	0.39	45	12.3
S-8	355	2.00	1.35	0.38	45	12.7
S-9	350	2.15	1.30	0.37	44	12.6
S-10	330	2.42	1.35	0.41	45	13.6
X	349.0	2.04	1.34	0.39	43.6	12.50
S.D.	12.94	0.26	0.05	0.02	2.61	0.93
S.E.M.	5.79	0.11	0.02	0.01	1.17	0.37
B-6	320				43	13.4
B-7	310				43	13.9
B-8	310				43	13.9
B-9	295				40	13.6
B-10	345				44	12.8
X	316.0[b,c]				42.6	13.52
S.D.	18.5.				1.52	0.46
S.E.M.	8.28				0.68	0.20

F = flight group
S = synchronous control group
V = vivarium control group
B = basal control group
Wt. = weight
B.W. = body weight

a = versus V, $p < 0.01$
b = versus S, $p < 0.01$
c = versus V, $p < 0.05$
d = versus V, $p < 0.001$
e = versus S, $p < 0.001$
f = versus B, $p < 0.001$

TABLE 2. PINEAL CONTENT OF RATS FLOWN ABOARD COSMOS 1887: MELATONIN (Mel), SEROTONIN (5-HT), 5-HYDROXYINDOLEACETIC ACID (5-HIAA), and CALCIUM (Ca).

Group-subj.#	Mel (pg/gl)	Mel (pg/mgt)	5-HT (ng/gl)	5-HT (ng/mgt)	5-HIAA (ng/gl)	5-HIAA (ng/mgt)	Ca (ug/gl)	Ca (ug/mgt)
F-6	121.9	80.8	23.103	15.300	19.306	12.785	1.02	0.68
F-7	112.9	59.4	20.228	10.646	14.740	7.759	0.95	0.50
F-8	37.6	27.1	24.302	17.610	17.404	12.612	1.12	0.81
F-9	28.2	11.4	8.594*	13.479*	6.989*	2.830*	1.09	0.44
F-10	37.5	25.9	15.669	10.806	12.893	8.892	0.42	0.30
X	67.62	40.92	20.83*a	13.59*a,b	15.09	10.51*a,b	0.92	0.55
S.D.	45.71	28.37	3.84	3.44	2.84	2.57	0.29	0.20
S.E.M.	20.44	12.69	1.92	1.72	1.42	1.28	0.13	0.09
V-6	50.3	31.0	20.810	12.846	11.912	7.353	ex	ex
V-7	57.8	30.1	12.715	6.622	7.693	4.007	2.76	1.44
V-8	92.3	50.3	14.956	8.128	17.079	9.282	1.37	0.74
V-9	176.7	83.8	15.368	7.283	9.032	4.281	0.92	0.44
V-10	12.4	5.5	18.299	8.169	11.664	5.207	0.93	0.42
X	77.9	40.1	16.43	8.61	11.48	6.03	2.06	0.53
S.D.	62.10	29.13	3.15	2.45	3.60	2.25	0.20	0.18
S.E.M.	27.77	13.03	1.41	1.10	1.61	1.00	0.12	0.10
S-6	87.8	47.2	27.878*	14.988*	54.805*	29.465*	0.99	0.53
S-7	144.0	82.3	13.419	7.668	15.361	8.778	0.98	0.56
S-8	31.2	15.2	11.169	5.845	4.358	2.179	0.90	0.45
S-9	90.8	41.8	13.390	6.228	10.080	4.688	0.97	0.45
S-10	65.7	27.1	10.875	4.493	6.617	2.734	ex	ex
X	83.9	42.72	12.15*	6.06*	9.10*	4.60*	0.96	0.50
S.D.	41.18	25.44	1.31	1.31	4.79	2.99	0.04	0.06
S.E.M.	18.42	11.38	0.65	0.65	2.40	1.50	0.02	0.02

F = flight group
S = synchronous control group
V = vivarium control group
Mel = melatonin
5-HT = serotonin
5HIAA = 5-Hydroxyindoleacetic acid
Ca = calcium
/gl = /whole pineal gland
/mgt = /milligram pineal tissue
ex = sample contaminated
a = versus S,p <0.01
b = versus V,p <0.05
* = means calculated without questionable values, see results

C5

TABLE 3. CONCENTRATION OF PLASMA SEROTONIN (5-HT), and TESTOSTERONE* OF RATS

Group-subj.#	5-HT (ng/mL)	Testosterone* (ng/mL)
F-6	11.87	0.18
F-7	7.88	0.18
F-8	17.21	0.62
F-9	7.89	0.10
F-10	N.D.	0.62
X	11.21[d]	0.34[a]
S.D.	4.42	0.26
S.E.M.	2.21	0.11
V-6	108.29	2.50
V-7	N.D.	1.20
V-8	28.06	0.43
V-9	38.14	0.48
V-10	14.99	0.24
X	47.37	0.97
S.D.	41.70	0.93
S.E.M.	20.85	0.42
S-6	20.72	0.62
S-7	N.D.	3.50
S-8	11.77	0.85
S-9	11.35	1.85
S-10	34.55	0.77
X	19.60[d]	1.52
S.D.	10.87	1.21
S.E.M.	5.43	0.54
B-6	30.05	4.50
B-7	47.88	4.20
B-8	44.87	3.40
B-9	73.50	2.40
B-10	123.99	2.00
X	64.06	3.30[b,c]
S.D.	36.97	1.09
S.E.M.	16.53	0.49

F = flight group
S = synchronous control group
V = vivarium control group
B = basal control group
N.D. = not determined

a = versus B, $p < 0.001$
b = versus S, $p < 0.01$
c = versus V, $p < 0.01$
d = versus B, $p < 0.05$
* = determined by RIA, data provided by Dr. R. Grindeland and M. Vasques, NASA-Ames

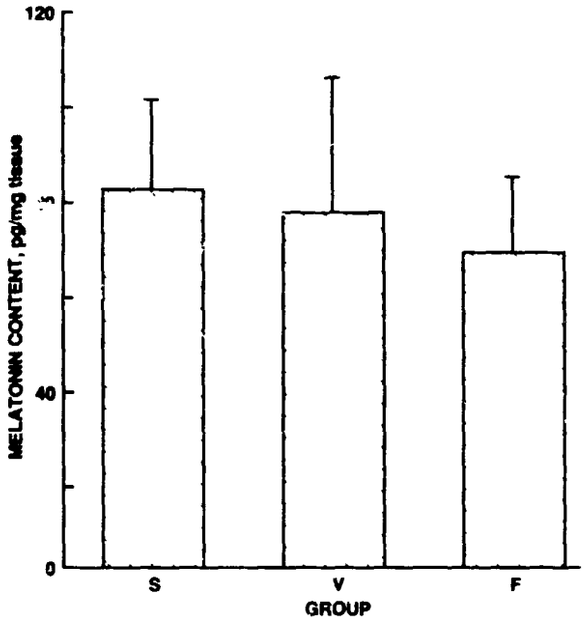

Figure 1. Pineal melatonin content (pg/gland) synchronous control group (S), vivarium control group (V), and flight animals aboard Cosmos 1887 (F). Values are means ± S.E.M., N = 5/group.

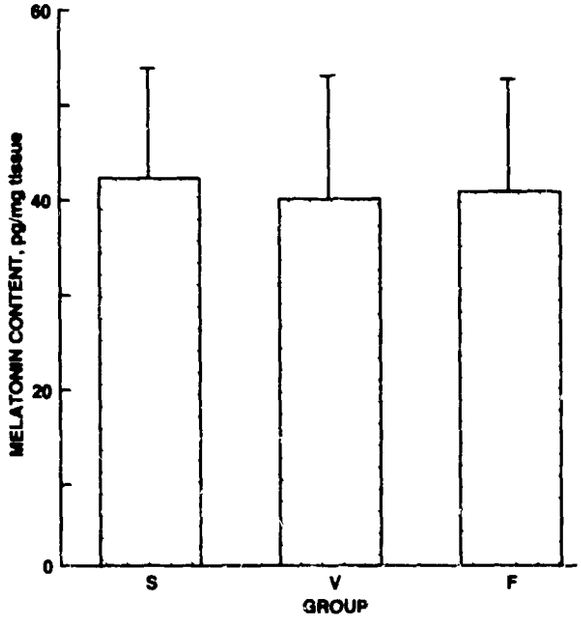

Figure 2. Pineal melatonin content (pg/mg tissue) synchronous control group (S), vivarium control group (V), and flight animals aboard Cosmos 1887 (F). Values are means ± S.E.M., N = 5/group.

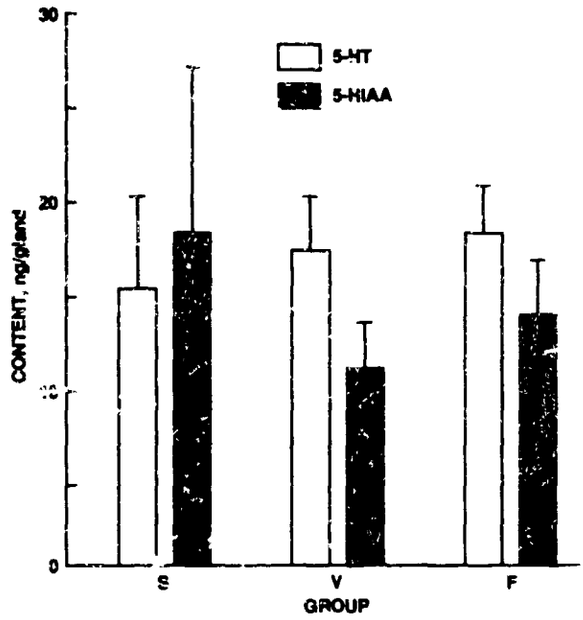

Figure 3. Pineal serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) expressed as ng/gland in synchronous control group (S), vivarium control group (V), and flight animals aboard Cosmos 1887 (F). Values are means ± S.E.M., N = 5/group.

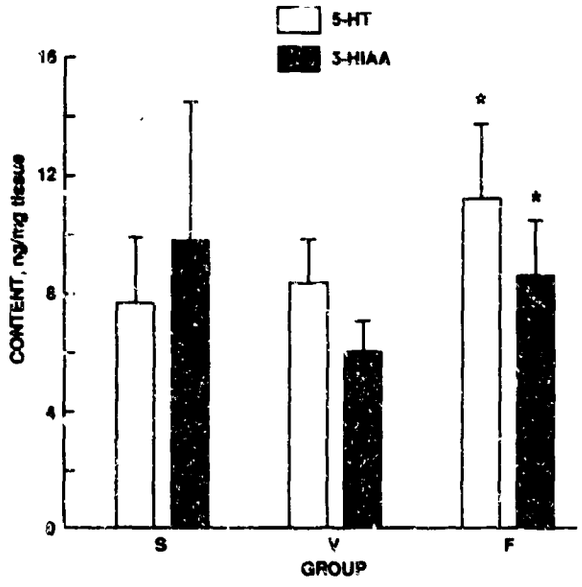

Figure 4. Pineal serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) expressed as ng/mg tissue, in synchronous control group (S), vivarium control group (V), and flight animals aboard Cosmos 1887 (F). Values are means ± S.E.M., N = 5/group.

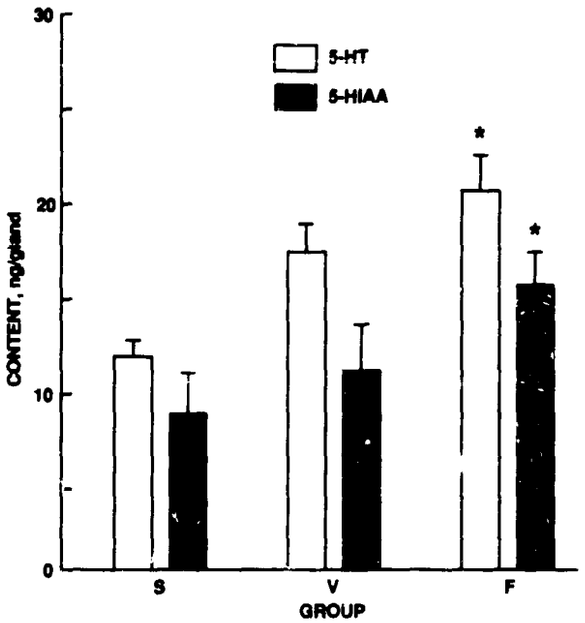

Figure 5. Pineal serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) expressed as ng/gland in synchronous control group (S), vivarium control group (V), and flight animals aboard Cosmos 1887 (F). Samples F-9 and S-6 were excluded from the analysis (see results section). Values are means ± S.E.M., N = 5 for V group, N = 4 for F and S groups. * = significantly different versus control, $p < 0.05$.

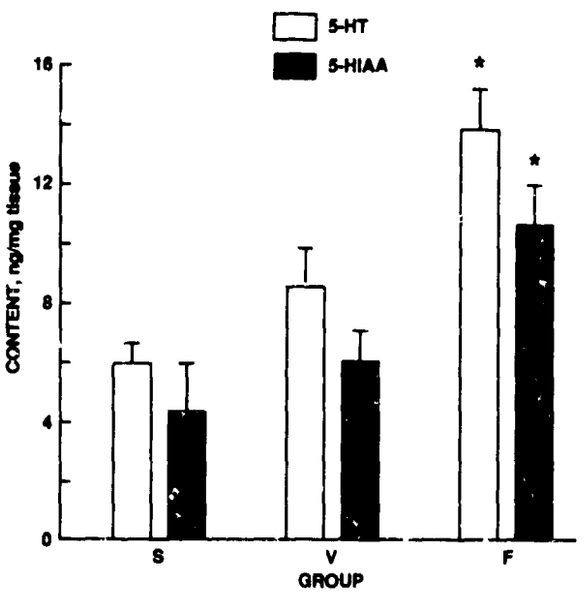

Figure 6. Pineal serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) expressed as ng/mg tissue, in synchronous control group (S), vivarium control group (V), and flight animals aboard Cosmos 1887 (F). Samples F-9 and S-6 were excluded from the analysis (see results section). Values are means ± S.E.M., N = 5 for V group, N = 4 for F and S groups. * = significantly different versus control, $p < 0.05$.